

Utilization of *Gracilaria chilensis* (Rhodophyta: Gracilariaceae) as a Biofilter in the Depuration of Effluents from Tank Cultures of Fish, Oysters, and Sea Urchins

FUNGYI CHOW, JUAN MACCHIARELLO¹, SERGIO SANTA CRUZ, ERIKA FONCK,
AND JORGE OLIVARES

*Facultad de Ciencias del Mar, Universidad Catolica del Norte, Casilla 117,
Coquimbo, Chile*

Abstract.—An evaluation was made on a seasonal basis of the effect of the marine macroalga culture *Gracilaria chilensis* on concentrations of some soluble and particulate wastes emitted during tank cultures of a fish *Isacia conteptionis*, an oyster *Crassostrea gigas*, and a sea urchin *Loxechinus albus* species. The animals were each cultured in separate tanks, and effluent from each was directed through separate tanks, which contained dense cultures of the *Gracilaria chilensis*. Inflow-outflow monitoring was conducted for the presence of nitrate, nitrite, ammonium, and phosphate. Also evaluated were particulate nitrogen and phosphate. The most significant wastes were ammonium from the fish culture and nitrate from the oyster culture. These were completely removed from the water, with minor exceptions, by the alga during all seasons of the year. Lesser amounts of soluble phosphate and nitrite, arising from the fish cultures, were also removed. Of the particulate matter, only nitrogen was in evidence from the fish cultures in the summer. It was concluded that *Gracilaria chilensis* culture was highly efficient at biofiltration of the soluble nutrients tested but had little effect on particulate emissions. The best growth of *Gracilaria chilensis* occurred in the ammonium-rich effluent from the fish culture.

Intensive aquaculture, where valuable marine species are cultivated under semi-artificial conditions in land-based seawater tanks, has undergone notable development in Chile, as in the rest of the world. This type of activity has experienced some criticism regarding its environmental impact due to its tendency to release waste effluents containing elevated levels of nitrogen or phosphorus-rich compounds (Cancino and Orellana 1987; Buschmann et al. 1994) which may be considered as water pollutants (Carefoot 1977). Since marine algae may have high capacities for absorption and

metabolism of N and P-rich compounds excreted by marine animals, and may grow in the process, a number of suggestions have been made for combining algae into mixed culture systems with vertebrates or invertebrates (Ryther et al. 1975; Langston et al. 1977; Ambler et al. 1988; Cohen and Neori 1991). *Gracilaria chilensis* Bird, McLachlan and Oliveira is a commercially valuable marine alga harvested at many locations on the Chilean coast for its content of agar. At present, 84% of Chile's production of *Gracilaria* comes from managed culture in the sea (Avila and Seguel 1993), although research has been conducted on the as yet unresolved possibility of its mass culture in tanks of seawater (Oliveira 1984). In the ideal situation, a commercially valuable alga such as *Gracilaria* may be used as a biofilter for nutrient-rich effluent, thus eliminating possible eutrophication and producing a valuable by-product in the process.

Since we had experimented with the culture of *Gracilaria chilensis* (Edding et al. 1987; Macchiavello et al. 1987; Macchiavello 1990), it was of interest to integrate our algal cultures into other projects at the Coastal Aquaculture Center that were studying the culture of fish, oysters, and sea urchins, thereby providing a biofiltration system for their effluents, and potentially benefiting the algae.

Materials and Methods

Our work was carried out in the Coastal Aquaculture Center and Marine Research of the Universidad Catolica del Norte, Co-

¹ Corresponding author.

quimbo, Chile (30°S, 71°W) between autumn of 1995 and summer of 1996. Culture effluents were obtained from: a local fish species *Isacia conceptionis* Cuvier (Perciformes, Haemulidae); the Japanese oyster *Crassostrea gigas* Thunberg (Mollusca: Bivalvia); and the local red sea urchin *Loxechinus albus* Molina (Echinodermata: Echinoidea). Animal cultures were each carried out in 625-L fiberglass tanks with a surface area of about 1.5 m². The tanks were outside, shielded from excess sunlight by black polyethylene shade cloth (20% transmission), and received an hourly seawater flow about equal to the total tank volume. Seawater for the experiments was obtained from Herradura Bay, adjacent to the laboratory, where it was filtered to about 60 µm through underwater sand filters and an above ground gravity filter (Giken Kosho Co., model G-K 40; Edding et al. 1987). The fish culture was stocked with individuals from 20.5 to 29.5 cm length at a density of about 12 kg/m³ and were fed daily with number 4 trout pellets (Aguas Claras S.A., Santiago, Chile), representing about 1% of the weight of the fish in culture. The oyster culture contained individuals of 104 to 162 cm length at a density of about 42 kg/m³, and were fed daily with 60 L of microalga culture *Chaetoceros gracilis* containing 1-2 X 10⁶ cells/mL. The urchin culture contained individuals of 31 to 78 mm test diameter at density of about 23 kg/m³, which were fed weekly with about 4 kg of the brown seaweed *Lessonia trabeculata*.

Effluent seawater was accumulated from cultures in 500-L closed cylindrical tanks, and a constant flow of water was passed on to the tanks of *Gracilaria chilensis*. Effluent flows of 50 L/h were directed through each of three replicate tanks of about 200 L with a surface area of 0.5 m² (Fig. 1). These tanks, acting as biofilter, each contained about 7 kg/m² of *Gracilaria chilensis* that had been collected in Herradura Bay near the laboratory, and acclimated in laboratory tanks for 30 d prior to experimentation. The growth of the algae was measured every 15

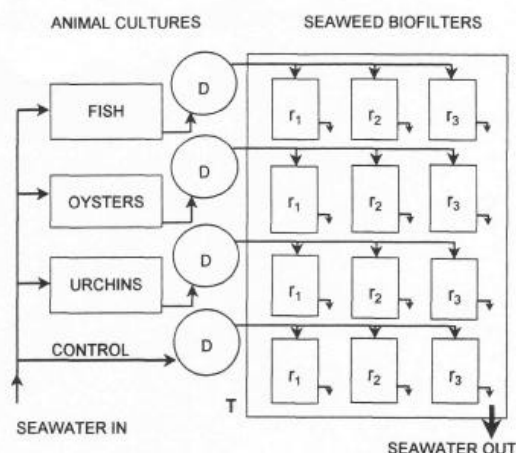


FIGURE 1. Schematic representation of the seaweed biofilter system. Arrows indicate water flows, D = decantation tank, r = replicate, T = thermoregulation pond.

d and expressed in productivity (g/m² per d) and relative growth rate: $RGR = (\log_2 W_f/W_0)/t$, where W_f = final wet weight in grams; W_0 = initial wet weight in grams, and t = time in days (Monod 1949). Excess algae resulting from growth was removed every 15 d. All tanks were submerged to the waterline in a 4 X 3 m pool, 0.6-m deep, which received seawater from the biofilters. Tanks were maintained at a similar, stable temperature. The minimum temperature in winter was 14 C, and the maximum in summer was 20 C. Culture tanks were continuously aerated with compressed air from air stones. At the beginning of each season, all visible epiphytes were removed from the *Gracilaria*, and the tanks were cleaned. Water samples for analysis were collected at inflow and outflow sites from all *Gracilaria* tanks, including a set of controls that received no animal effluents at intervals of about 20 d. Quantitative analysis was done on all water samples for NO₃⁻, NO₂⁻, NH₄⁺, PCV³, particulate N, and par-ticulate P. Soluble nutrients were determined by the methods of Strickland and Parsons (1972), with results expressed as (µmol/L. Particulate N and P were determined spectrophotometrically after filtration of 250-mL samples on 0.45 µm mem-

brane filters using the Kjeldhal method modified by Branstreet (Walton 1970) and Strickland and Parsons (1972), respectively. Three sets of chemical analyses were carried out per season at intervals of about 20 d.

All data were analyzed using SYSTAT 8.0 Software (SPSS Inc. Chicago, Illinois, USA). Dates were tested for normality and homoscedasticity before applying parametric or non-parametric tests as appropriate. Data on effluent from cultured animals was analyzed for season and between seasons by ANOVA or non-parametric Kruskal-Wallis test (Zar 1984). When differences for season from cultured animals were significant ($P < 0.05$), a Dunnett comparison test (or its corresponding non-parametric test; Zar 1984) was made to determine if amounts of nutrients exiting were significantly different from control seawater. To differentiate between seawater inflow and outflow from the *Gracilaria* tanks (biofilter) we applied a Student Mest.

Results

Seasonal values for N and P compounds in cultured animal effluents are shown in Fig. 2. Although some variation is seen in these results, the only increments that were statistically significant were those for ammonium in the fish culture during all four seasons (Fig. 2c), and nitrite (Kruskal-Wallis, $P < 0.05$ and non-parametric Dunnett test, $P = 0.05$) and soluble P during autumn (ANOVA, $P < 0.05$ and Dunnett test $P = 0.05$) (Figs. 2b, 2d). Also, the oyster culture released a significant amount of nitrate during the winter (ANOVA, $P < 0.05$ and Dunnett test $P = 0.05$) (Fig. 2a). The effluent from the urchins showed some increase in phosphate, but this was not statistically significant at any time of the year (ANOVA, $P > 0.05$ and Dunnett test $P = 0.05$). Release of particulate N was significant from the fish culture during the summer (ANOVA, $P < 0.05$ and Dunnett test $P = 0.05$) (Fig. 2e), and particulate P did not increase significantly for any of the cultures (ANOVA, $P > 0.05$). When we compared increases in

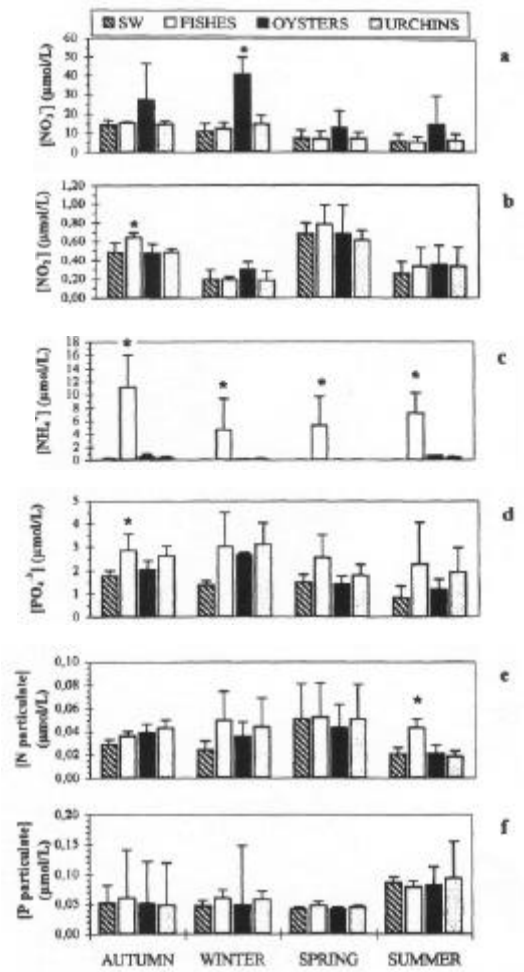


FIGURE 2. Seasonal concentrations of chemical nutrients in effluents from fish, oyster, and sea urchin cultures as described in text. Data are presented as means (bars) \pm SEM, $N = 3$. Significant difference ($P < 0.05$) from Bay seawater (control) are indicated by an asterisk above the standard error bar.

N and P compounds released by the marine animal cultures over the entire study period, only particulate N released by the fish culture showed a significant seasonal variation (Kruskal-Wallis, $P = 0.0469$).

The *Gracilaria* tanks acted effectively as biofilters throughout the entire year, as they were able to absorb most of the soluble nutrients released by the animal cultures (Fig. 3). When nutrients from incoming water were summed with nutrients contributed

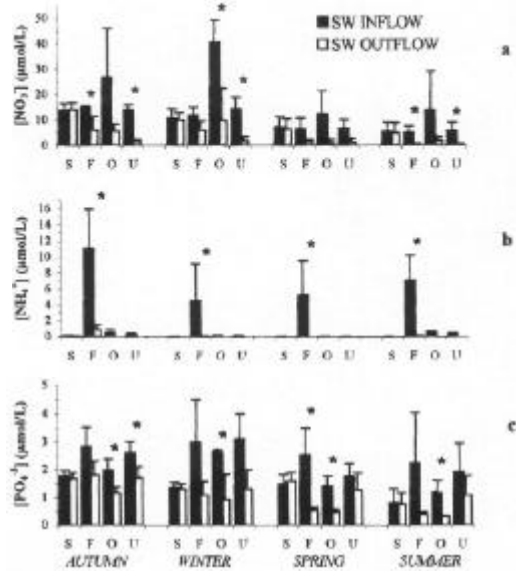


FIGURE 3. Seasonal concentrations of dissolved chemical nutrients in seawater entering and exiting *Gracilaria* biofilters, with culture effluent obtained from fish (F), oyster (O), sea urchin (U) cultures and seawater control (S). Data are presented as means (bars) \pm SEM. Significant differences (Student *t*-test, α 0.05) between seawater entering and exiting are indicated by asterisk above the standard error bar.

from animal cultures, significant (Student *t*-test, α 0.05) lowering of nutrients by the biofilter was observed for the ammonium from the fishes all year and for nitrate from the oysters in winter. Some of the inflow variables (nutrients) that were not significantly augmented by the animal cultures were still relatively high due to enrichment of the incoming seawater, and were removed by the *Gracilaria* cultures. This was particularly significant (Student *t*-test, α 0.05) in removal of nitrate coming from the fish culture in autumn and summer, nitrate from urchins during autumn, winter and

summer (Fig. 3a), phosphate from the fishes during spring, from the oysters all year, and from the urchins during the autumn (Fig. 3c).

Table 1 shows the values for average annual productivity and relative growth rate (RGR) for *Gracilaria* in different effluents. Both productivity and RGR showed similar tendencies, where the growth in the fish effluent was three times greater than the control value, and also greater than in effluents from the urchins and oysters.

Discussion

General Considerations

The present year-long experiment demonstrated that a macroalgal culture may be coupled to an intensive aquaculture system, producing a valuable by-product and reducing otherwise contaminating nutrients in the effluent. Our seawater flow amounted to over 10,000 L/d, which represented a significant proportion of all the seawater pumped daily by the Coastal Aquaculture Center (CAC). Mortalities of animals in culture were negligible, and the three types of organisms represented some of the various activities in progress at the CAC. Animal densities in the tanks were typical of those used in experimentation at the CAC. Manipulation of effluent flows and nutrient concentrations was not within the scope of the first year of this study, although our results suggested that in the future effluent flows could be increased substantially without loss of efficiency of the biofilter, especially in cases where quantities of nutrient in the effluent were low (e.g., urchins). Yield of animal products grown in the system was good, however, these data are be-

TABLE 1. Average annual productivity (fresh weight, g/m² per d \pm SEM) and relative growth rate (RGR) of *Gracilaria chilensis* in effluents from cultures of fish, oysters and sea urchins.

Effluent type	Fish	Oysters	Urchins	Control
Productivity	51.3 \pm 25.1	23.9 \pm 17.1	16.2 \pm 2.4	18.6 \pm 13.6
RGR	0.010	0.004	0.003	0.004

yond the scope of this report. As mentioned above, the best growth of *Gracilaria* was obtained with the ammonium-rich effluent from the fishes, as this nutrient was rapidly absorbed by the alga, in agreement with Cancino and Orellana (1987) and Ambler et al. (1988). Maximum productivity obtained in the present study (51.3 g/m² per d) was somewhat lower than that obtained in a comparable experimental culture of *Gracilaria* (62.2 g/m² per d; Edding et al. 1987) although water flow in the former (50 L/h) was slower than in the latter (200 L/h). Since a major cost in *Gracilaria* and other macroalgal cultures is that of providing large water flows, the economy of using effluent flows from animal cultures appears attractive, especially since the present results suggest that water flow may be increased several-fold through the *Gracilaria* culture biofilter without loss of nutrient-stripping efficiency.

Nutrients in Effluents

The significant emission of ammonium from the fish culture was expected, as reported for other fish cultures by Krom et al. (1985), Cohen and Neori (1991) and Buschmann et al. (1994). The minor amount of ammonium released from the oyster culture was low compared with results of other authors (Poblete et al. 1991), and with other bivalves, such as *Argopecten purpuratus* (Ambler et al. 1988) and *Mytilus chilensis* (Retamales and Buschmann 1996), although these differences may be due to differences in experimental design or water flow rates. Nutrients coming from the oysters may have reflected nitrate and phosphate in the microalgal cultures given to the oysters as food, particularly in winter when the oysters had lower rates of feeding activity.

In a complex system such as the one described, there were several variables that went uncontrolled and must be taken into consideration in future research. Primary among these are levels and activities of microorganisms in the system, as these may

affect the observed results by decomposing organic residues to release ammonium, oxidizing ammonium to nitrite and nitrate, and converting soluble organic and nutrients to particulate matter. Further work must take into account the possibility of regulating the flows of effluent to the *Gracilaria* culture in relation to their nutrient load as determined by the type of animal under cultivation, and by season of the year, particularly in temperate climates.

In conclusion, the practical value of our system seems to have been demonstrated by using *Gracilaria chilensis* culture as an effective biofilter. Long-term studies will be required to determine optimal flow rates, organism loading, and economically viable system design.

Acknowledgments

This project was supported by the Subsecretaria de Pesca del Ministerio de Economía, Fomento y Reconstrucción de Chile. We thank Danilo Peralta and Sergio Fuentes for his help in the set up of the experiments. Dr. Louis DiSalvo helped to improve the manuscript.

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